

Appl. No. 09/262,126
Amdt. dated November 4, 2004
Reply to Office action of May 4, 2004

Page 2

IN THE CLAIMS:

The claims as currently presented and under consideration, are presented below for the Examiner's convenience and to comply with 37 CFR §1.121. This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-4 (Cancelled)

5. (Previously amended) The pullulanase of Claim 6, wherein the pullulanase is obtained from a *Bacillus deramification* having the designation T89.117D in the LMG culture collection.

6. (Previously amended) A truncated *Bacillus* pullulanase comprising a deletion of about 100 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

7. (Previously amended) A truncated *Bacillus* pullulanase comprising a deletion of about 200 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

8. (Previously amended) A truncated *Bacillus* pullulanase comprising a deletion of about 300 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

9. (Previously Amended) A truncated *Bacillus* pullulanase comprising a deletion that is 98 amino acids from the amino terminus of *Bacillus deramificans* pullulanase, wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

10. (Previously Amended) A truncated *Bacillus* pullulanase comprising a deletion that is 102 amino acids from the amino terminus of *B. deramificans* pullulanase, wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,

Appl. No. 09/262,126
Amdt. dated November 4, 2004
Reply to Office action of May 4, 2004

Page 3

6-glucosidic bond.

11. (Cancelled)

12. (Previously amended) A modified *Bacillus* pullulanase which is capable of hydrolysis of an alpha-1,6-glucosidic bond, wherein the modification is an addition of at least one amino acid to the amino terminus of a mature pullulanase amino acid sequence obtainable from a *Bacillus deramificans*, wherein the additional amino acid at the amino terminus is an alanine.

13. (Cancelled)

14. (Currently Amended) A truncated *Bacillus* pullulanase produced by a method comprising the steps of

a) obtaining a recombinant host cell comprising nucleic acid encoding a mature *Bacillus* pullulanase said nucleic acid having at least 90 % identity to the polynucleotide sequence as shown in SEQ ID NO:1,

b) culturing said host cell under conditions suitable for the production of a truncated pullulanase, and

c) recovering the truncated pullulanase wherein the truncated *Bacillus* pullulanase comprises a deletion of about 100 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

15. (Previously Amended) The pullulanase of Claim 14 wherein said host cell is *B. licheniformis* which comprises a first gene encoding Carlsberg protease and a second gene encoding endo Glu C protease, the first and/or second gene which codes for the protease(s) having been altered such that the protease activity is essentially eliminated.

Claims 16 - 26 (Cancelled)

Appl. No. 09/262,126
Amdt. dated November 4, 2004
Reply to Office action of May 4, 2004

Page 4

27. (Previously amended) An enzymatic composition comprising a truncated *Bacillus deramificans* pullulanase wherein said truncated pullulanase is selected from the group of pullulanases consisting of

- a) a deletion of up to about 100 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase,
- b) a deletion of up to about 200 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase, and
- c) a deletion of up to about 300 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase,

wherein said truncated pullulanase of a), b) or c) comprises a conserved Y position and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

28. (Previously Amended) The enzymatic composition of Claim 27 wherein the truncated pullulanase has a deletion of amino acids from the amino terminus of up to about 100 amino acids.

29. (Previously Amended) The enzymatic composition of Claim 27 wherein the truncated pullulanase has a deletion of amino acids from the amino terminus of up to about 200 amino acids.

30. (Previously Amended) The enzymatic composition of Claim 27 wherein the truncated pullulanase has a deletion of amino acids from the amino terminus of up to about 300 amino acids.

31. (Previously Amended) An enzymatic composition comprising the pullulanase of Claim 9, wherein the pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 99, a glutamic acid.

32. (Previously Amended) An enzymatic composition comprising the pullulanase of Claim 10, wherein the pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 103, a glutamic acid.

33. (Original) The composition of Claim 27 further comprising an enzyme

Appl. No. 09/262,126
Amdt. dated November 4, 2004
Reply to Office action of May 4, 2004

Page 5

selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.

34. (Original) The composition of Claim 27 further comprising a glucoamylase.

35. (Original) The composition of Claim 34 wherein the glucoamylase is obtainable from an *Aspergillus* strain.

36. (Original) The composition of Claim 35 wherein the *Aspergillus* strain includes *Aspergillus niger*, *Aspergillus awamori* and *Aspergillus foetidus*.

37. (Original) The composition of Claim 27 wherein said composition is in a solid form.

38. (Original) The composition of Claim 27 wherein said composition is in a liquid form.

39. (Currently Amended) The composition of Claim 27 comprising at least 60% ~~modified~~ truncated pullulanase.

40. (Currently Amended) The composition of Claim 27 comprising at least 80% ~~modified~~ truncated pullulanase.

Claims 41 to 51 (Cancelled)

52. (Previously Added) The truncated *Bacillus* pullulanase of claim 6, wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

53. (Previously Added) The truncated *Bacillus* pullulanase of claim 7, wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

Appl. No. 09/262,126
Amdt. dated November 4, 2004
Reply to Office action of May 4, 2004

Page 6

54. (Previously Added) The truncated *Bacillus pullulanase* of claim 8, wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

55. (Previously Amended) The enzymatic composition of claim 27 wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

56. (Previously Amended) The truncated *Bacillus pullulanase* produced according to the method of claim 14, wherein the nucleic acid sequence encoding the mature pullulanase is SEQ ID NO: 1.

57. (Previously Amended) The truncated *Bacillus pullulanase* produced according to the method of claim 14, wherein the mature pullulanase has the sequence shown in SEQ ID NO: 2.

58. (Previously added) The truncated *Bacillus pullulanase* of claim 9, wherein the pullulanase is obtained from a *Bacillus deramificans* having the designation T89.117D in the LMG culture collection.

59. (Previously added) The truncated *Bacillus pullulanase* of claim 10, wherein the pullulanase is obtained from a *Bacillus deramificans* having the designation T89.117D in the LMG culture collection.

60. (Currently Amended) The truncated *Bacillus pullulanase* of claim 6, further comprising a conserved VWAP (SEQ ID NO:9) region.

61. (Currently Amended) The truncated *Bacillus pullulanase* of claim 7, further comprising a conserved VWAP (SEQ ID NO:9) region.

62. (Currently Amended) The truncated *Bacillus pullulanase* of claim 8, further comprising a conserved VWAP (SEQ ID NO:9) region.

Appl. No. 09/262,126
Amdt. dated November 4, 2004
Reply to Office action of May 4, 2004

Page 7

63. (Currently Amended) The truncated *Bacillus pullulanase* of claim 14, further comprising a conserved VWAP (SEQ ID NO:9) region.

64. (Currently Amended) The truncated *Bacillus pullulanase* of claim 27, further comprising a conserved VWAP (SEQ ID NO:9) region.

65. (Previously Added) The composition of Claim 31 further comprising an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.

66. (Previously Added) The composition of Claim 32 further comprising an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.